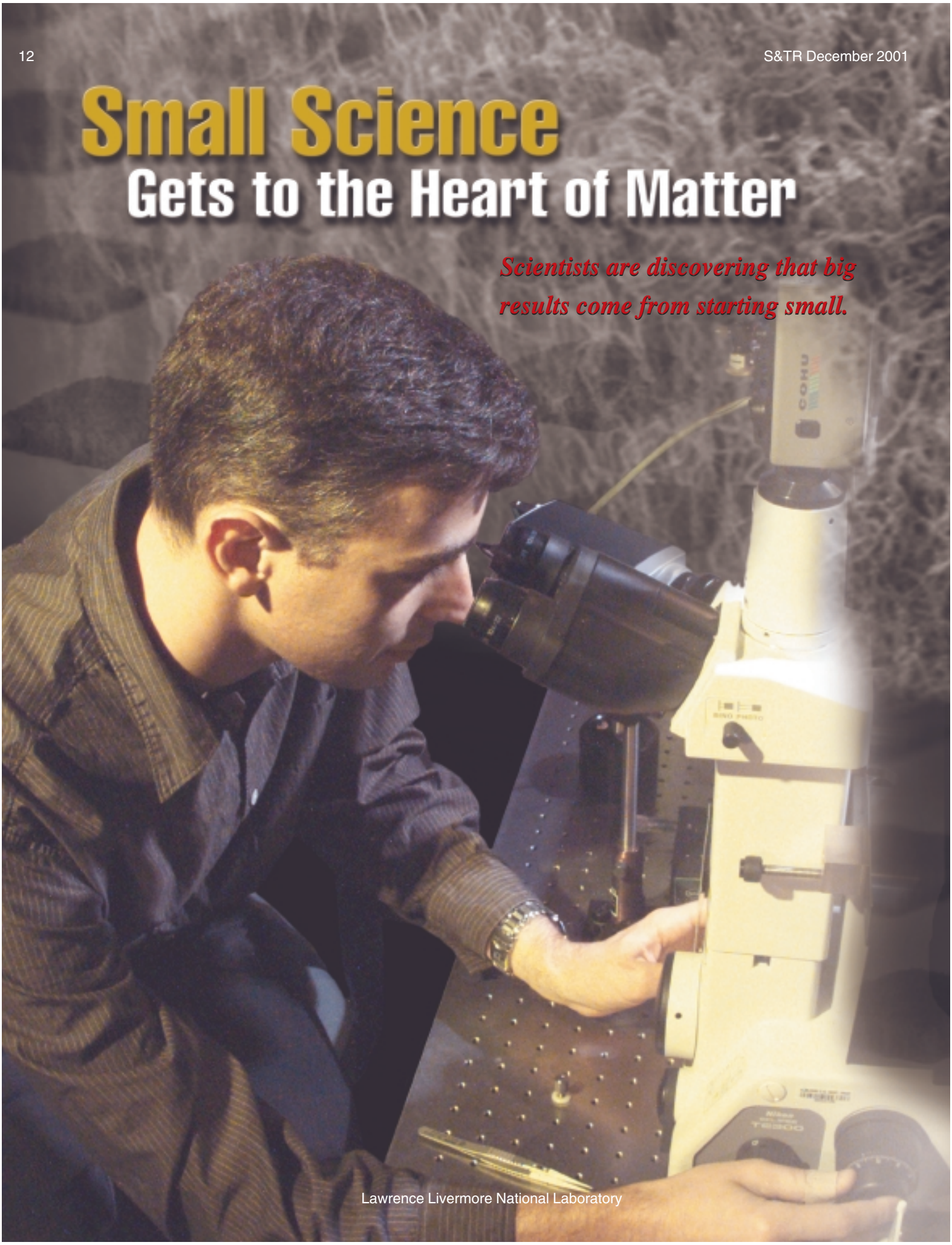
Small Science Gets to the Heart of Matter

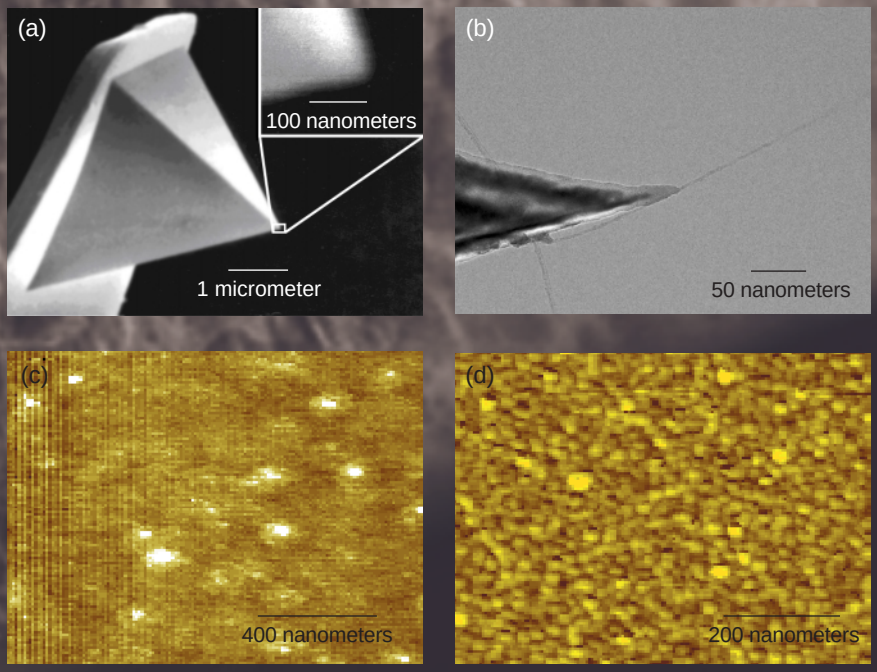
Scientists are discovering that big results come from starting small.



FINDING the best ways to detect biological warfare agents is one of Lawrence Livermore's missions today. Detecting large quantities of a biological pathogen is not difficult. The challenge is in detecting a few molecules of a toxin or a few bacteria or viruses to provide the early warnings of a biological attack.

Physicist Christine Orme and colleagues in the Chemistry and Materials Science Directorate are helping to understand some of the fundamental issues that underlie biodetection as well as fulfilling other Laboratory goals. They are performing research at minute scales in a field known as nanoscience, which takes its name from nanometer, a billionth of a meter. The team is examining, on an atom-by-atom and molecule-by-molecule basis, the organization of materials on surfaces and learning how that organization affects material properties. "One of the keys to working in nanoscience is controlling the surface and then being able to detect what is there," says Orme.

At the nanoscale, experimental results can be viewed only with the most powerful imaging tools. The atomic force microscope (AFM) has been used since the mid 1980s to produce topographic maps of nanostructures. Today, Orme's colleagues are developing new microscopic techniques based on use of the AFM that give even higher resolution and supply more than just topographic data. They are also refining the spectroscopic techniques that identify chemical bonds and supply fingerprints of molecules.



(a) Typical atomic force microscopy (AFM) tip and (b) nanotube tip. With the smaller nanotube tip, it is possible to obtain much more detailed information about a surface. AFM images of titanium grains obtained using (c) a typical AFM tip and (d) a nanotube tip.

The current research builds on pioneering Livermore work in crystal growth and thin multilayers, both of which depend on a keen understanding of material behavior at the atomic level. Livermore has a long-standing effort in crystal growth and characterization, born out of the need for large, ultrapure crystals in Livermore's lasers. Multilayers—exceedingly thin alternating layers of materials—were first demonstrated more than 50 years ago. But improved fabrication technologies developed by Livermore's Troy Barbee have prompted their use as highly reflective mirrors for telescopes as well as in a variety of optical applications, including electron microprobes, scanning electron microscopes, and particle beamlines in accelerators. (See *S&TR*, December 1999, pp. 11–13.)

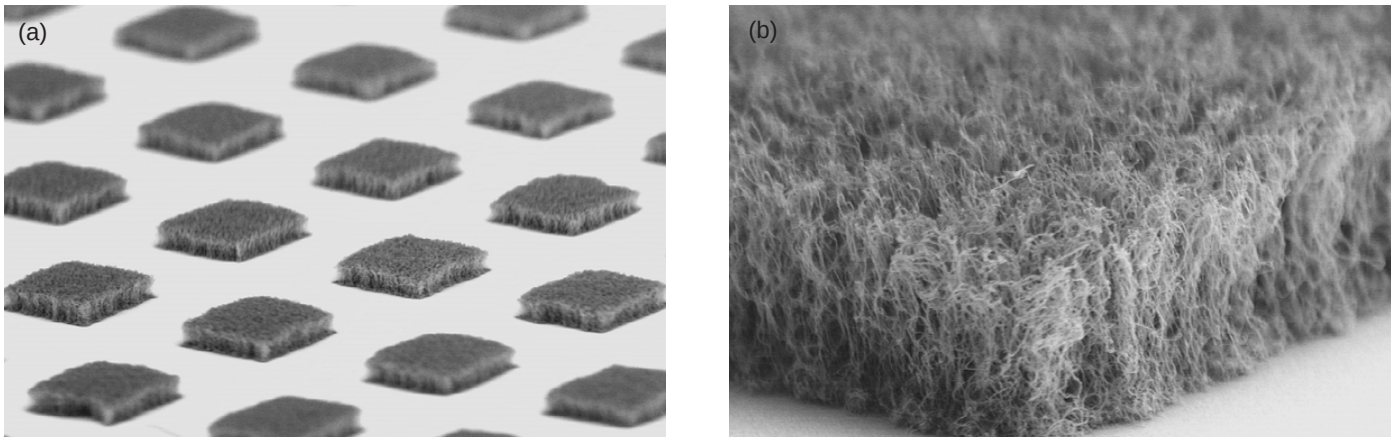
Seeing Is Believing

In atomic force microscopy, an extremely sharp tip senses the atomic shape of a sample while a computer records the path of the tip and slowly builds up a three-dimensional image. The AFM tip is positioned at the end of an extremely thin cantilever beam and touches the sample with a force of only 1/10-millionth of a gram, too weak to budge even one atom. As the tip is repelled by or attracted to the sample surface, the cantilever beam deflects. By imaging a larger or smaller area, researchers can vary the level of magnification of an AFM image. The

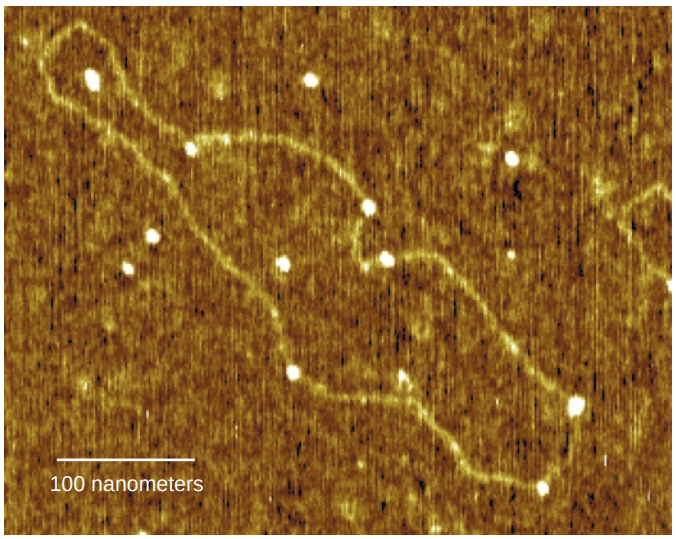
AFM can also be adapted to sense a range of forces including attractive or repulsive interatomic forces, electrostatic forces, and magnetic forces.

But even the sharp tip of the AFM is sometimes not tiny enough for the small scale at which the research team is working. Physical chemist Aleksandr Noy is growing carbon nanotubes that can be used to replace the standard AFM tip. The figure above compares a typical AFM tip and a carbon nanotube tip. Carbon nanotubes are built of carbon hexagons that are arrayed in a configuration resembling chicken wire. They are 1/50,000th of the width of a human hair but a hundred times stronger than steel at one-sixth the weight. Noy can make many kinds of nanotubes—single wall, multiwall, thick, thin, single isolated, or large arrays. The smaller, lighter nanotube tip tracks the shape of an object more accurately to provide more detailed information about its surface.

Noy used the nanotube-tipped AFM to image the cucumber mosaic virus and reveal its structure fairly clearly. AFM images contain less information than structures revealed through x-ray diffraction techniques, but Noy's image was captured in minutes, whereas the same structure took over a year to resolve from diffraction data. "In principle, this technology could be used to image a single virus," says Noy. "Emergency workers could compare its image with a



(a) "Farms" of carbon nanotubes and (b) a closeup of one farm. Livermore is exploring the potential of such nanotube arrays for detection applications.



One of the first images of DNA repair proteins bound to DNA.

Aleksandr Noy with the atomic force–confocal optical microscope.

computerized database of known virus structures to identify it very quickly."

With the nanotube tip on the AFM, a team led by Noy also obtained the first unambiguous visualization of a DNA repair protein bound to DNA. By incorporating a synthetic mutagenic molecule into DNA and tagging a repair protein with a fluorochrome, they will be able to study the repair process in situ.

Another imaging technique being used by physicist Thomas Huser and others is confocal microscopy. It is based on a fluorescence microscope augmented with a pinhole that limits the volume being probed to get rid of extraneous background "noise." Its beam can be focused to 500 nanometers. The confocal microscope efficiently collects fluorescence emitted from fluorescent molecules that have been excited by laser light. With this spectroscopic technique, Huser has been able to detect single molecules.

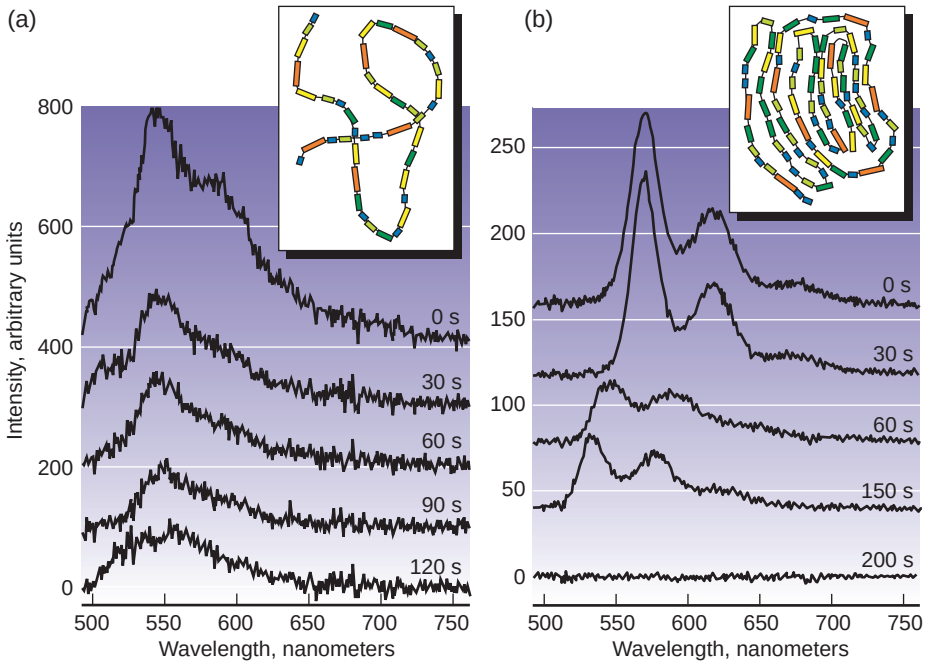
The confocal microscope is ideal for studying conjugated polymers, a new material that may be used to fabricate the next generation of light-emitting diodes (LEDs). Known as 2-methoxy, 5-(2′-ethyl-hexyloxy)-p-phenylene-vinylene, or MEH-PPV, the polymers are composed of a chain of benzene rings that emit light when linked by electrodes to which voltage is applied. The advantages of these polymers over the inorganic semiconducting materials of today's LEDs are many: They are easier to process on a large scale, they can be used to create ultrathin and flexible devices, and their power consumption is lower. Last year's Nobel Prize in Chemistry was awarded for the development of conjugated polymers.

Huser has learned that the physical configuration of the MEH-PPV molecules affects their fluorescence. "The photoluminescence of conjugated polymers depends strongly on how they are shaped," says Huser. When they fold up into a well-organized pattern in toluene, their shape enhances efficient energy transfer within the molecule. As conjugated polymers begin to be used as LEDs in electronics, some LED applications will take advantage of the high-energy-transfer configuration while others will benefit from the less ordered pattern for low-energy transfer.

In experiments, Huser exposed MEH-PPV to two solvents, toluene and chloroform. In toluene, the MEH-PPV molecules curl up tightly because, says Huser, "They don't like toluene. They try to avoid it." Spectrographic data collected every 5 seconds show a slight flicker as the molecules die off with exposure to oxygen and the light they emit shifts from red to blue. In chloroform, the polymer spreads out. There is no blue shift, the light spectrum is broader, and the light intensity simply decays slowly with time.

Huser recently began experiments with the confocal microscope to examine the dynamics of single molecules of DNA. Fluorescent labeling of DNA, RNA, enzymes, and proteins is common laboratory practice to illuminate the interactions and functions of these important biomolecules.

At the same time, Noy has built a whole new microscope system that combines the topographic capabilities of the AFM and the spectroscopy of the confocal microscope. He will be using this system to obtain even better information about DNA repair as well



The development of photoluminescence over time in the conjugated polymer MEH-PPV, a material with multiple fluorophor segments on a chain. (a) MEH-PPV exposed to chloroform forms an open, irregular coil (see inset) that leads to luminescence from multiple sites, hence the broad spectral emission. (b) MEH-PPV exposed to toluene forms a tight coil (see inset) with strong overlap between segments. In this conformation, only the segments with the lowest transition energy emit light. Thus, the emission is narrow and more structured. Once all the red fluorophors are photodestructed, the segments with the next lowest energy begin to emit light at slightly blue-shifted wavelengths.

as new information on how DNA is packaged.

Identifying a Single Molecule

Another tool for identifying molecular species is Raman spectroscopy, a form of light scattering similar to fluorescence. Although Raman-scattered light is much less intense than fluorescence, the technique is a powerful analytical tool because the changes in wavelength of the weakly scattered light are characteristic of the scattering material. Raman spectroscopy can identify chemical bonds and obtain the unique fingerprint of a molecule. Every molecule has a unique Raman spectrum, but not every molecule fluoresces. Raman spectroscopy is one of the few optical techniques that can identify a molecular species and determine its chemical bonding by observing its distinct molecular vibrational frequencies.

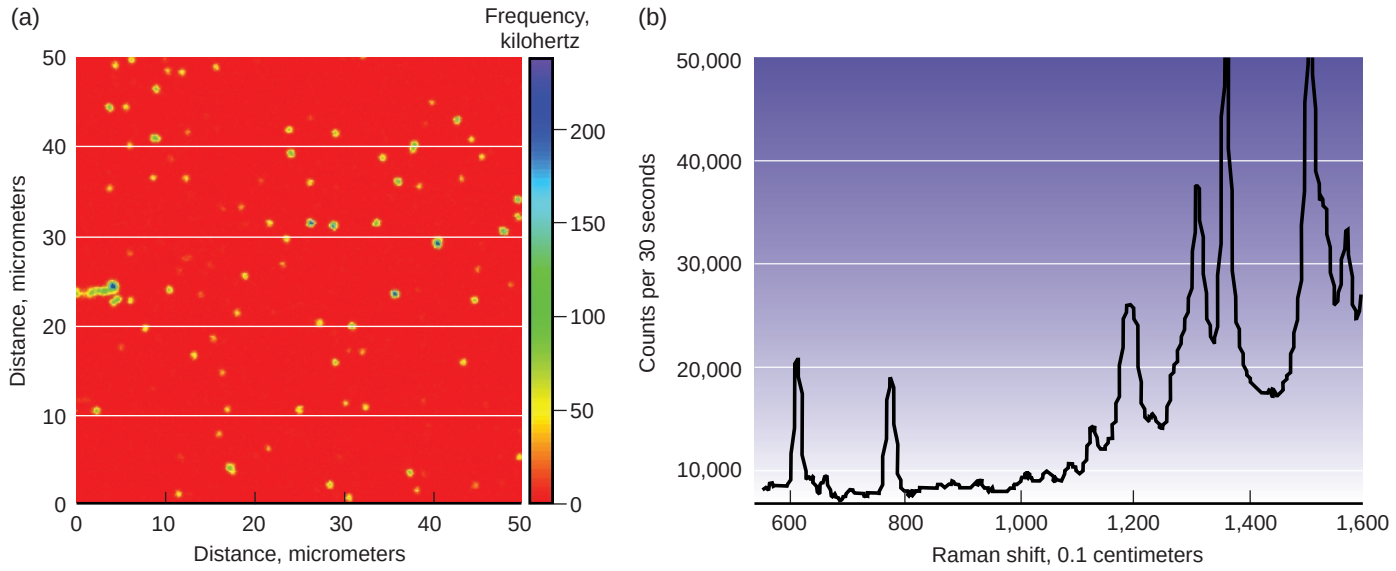
To increase the brightness and thus the resolution of Raman-scattered light, Huser has introduced nanometer-size gold crystals to the tip of a scanning probe microscope in a technique known as surface-enhanced Raman spectroscopy. The gold is negatively charged and attracts positively charged materials such as amino acids to adhere to kinks in the crystals. Electron density waves radiate from the corners of the gold crystals and increase the Raman signal by a factor of a quadrillion. At the same time, the scanning probe produces an image of the physical structure of the sample. The combined data allow for identification of single molecules. Unlike fluorescence, which fades with exposure to oxygen, the increased energy from the gold particles persists.

"Being able to characterize materials and chemical bonds at the level of a single molecule is a whole new capability for Livermore," says Huser. It is possible to perform Raman spectroscopy on single DNA molecules or proteins and to look for differences between individual cells. Using this technique, scientists also can detect and identify the byproducts or precursors of chemical agents such as the nerve gas sarin. This capability is important in the development of sensors for chemical warfare agents.

Controlling Biomolecules

Some nanoscience projects require the careful design of surfaces to collect and organize atoms, molecules, nanocrystals, colloids, cells, and spores. These surfaces are known as templates or, as Noy describes them, "landing pads" for toxins, proteins, and other biomolecules.

Livermore is exploring several techniques for creating templates. Physicist Jim De Yoreo is developing one method based on dip-pen nanolithography, which dips the tip of the AFM into an "inkwell" of organic molecules to "write" on an inorganic surface. As the tip moves across the surface, it makes a pattern that has almost no topographic relief but exhibits chemical contrast with the surrounding region. It is even possible to create multiple ink patterns with this method. The feature size is controlled by such factors as tip coverage, humidity, and contact time with the substrate, or, in the case of lines, tip



An example of the benefit of surface-enhanced Raman spectroscopy. (a) Confocal optical micrograph of 60-nanometer-diameter gold nanocrystals loaded with just a few molecules of the laser dye rhodamine 6G. (b) Surface-enhanced Raman spectrum of one of the gold particles in (a) easily identifies the adsorbed rhodamine by its characteristic Raman signature.

speed across the substrate. Examples of patterns created using a gold-coated mica surface for the substrate and 16-mercaptohexadecanoic acid for the ink are shown in the figure at right. This method has been used to deposit patterns of antibodies that would attract toxins and viruses, a first step in the development of nanostructured biosensors.

Another major area of research at Livermore's Biology and Biotechnology Research Program (BBRP) and elsewhere is in proteomics, the study of proteins. Cells produce particular proteins either all the time or as needed to prompt gene expression, that is, to turn a specific part of the genetic code on or off. Without proteins, our DNA could not operate properly. One of the best ways to examine the structure of a protein is to crystallize it and then subject it to x rays to obtain its unique diffraction pattern. During the crystallization process, molecules come together and separate (in a process known as nucleation) until a critical size is reached. Reaching that critical size can take a long time, and sometimes it does not happen at all. One goal of current proteomics work is to speed up the nucleation process and make it more likely that proteins will crystallize.

Dip-pen lithography, using a chemical that would prompt protein nucleation, is an option. "But," says Orme, "the size scale is a challenge. Proteins are extremely small, typically from 1 to 10 nanometers."

"If we make the pen's lines smaller, they won't be visible," adds Noy. So he and researchers in BBRP are developing a fluorescent ink for drawing lines with the density of a single molecule. In initial tests, a single-molecule line of the human chorionic gonadotropin (HCG) antibody has been successfully drawn. The next step will be to attract the HCG protein.

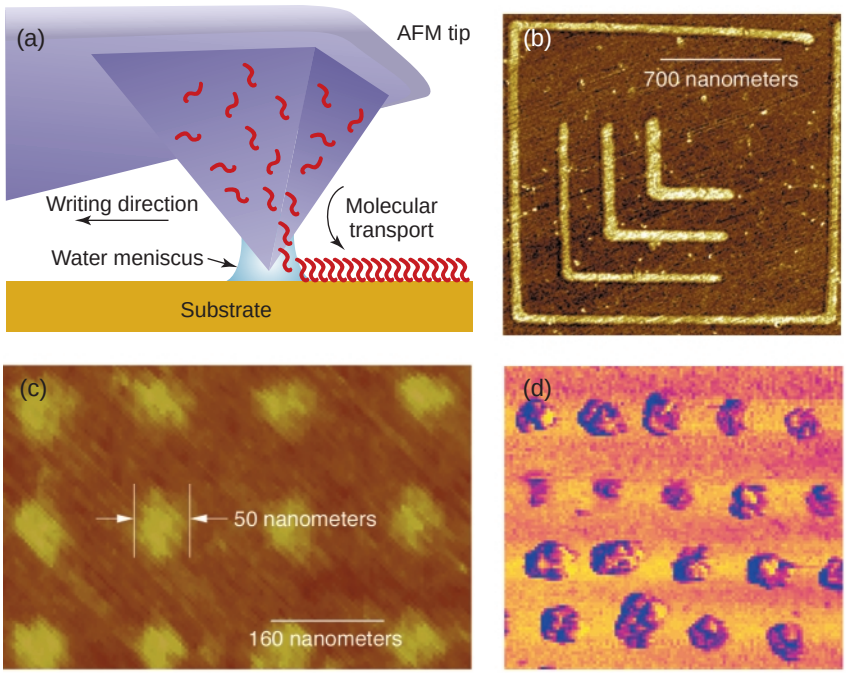
Nanolaminates, the next generation of multilayers, are also being explored as a way to accelerate the nucleation and growth of ordered proteins. Nanolaminate structures have been successfully synthesized with layers that are the same small size as typical proteins. The alternating layers have different surface charges, which prompt the proteins to adsorb in ordered rows. In the example shown in the top figure on p. 18, a nanolaminate was dipped into a solution of the protein ATCase. The nanolaminate was then removed, rinsed, air-dried, and imaged with AFM using a carbon nanotube tip. The resulting extremely high resolution of the image makes nonspherical proteins individually distinguishable on silica stripes. An image of the same deposition onto a homogeneous silica surface is very different, lacking any linear order. This set of experiments was the first step in accelerating nucleation and growing protein crystals that are suitable for x-ray diffraction.

Mimicking Natural Growth

Nanoscience is finding another application in the hands of Orme, De Yoreo, and colleagues whose research on the growth of calcite crystals sheds new light on the formation of bones, eggshells, and seashells.

The natural growth of organic crystals is known as biomineralization. Biomimetics is the term for mimicking nature's building methods to make a synthetic material. "We can only learn to make better bones and teeth if we first understand how the materials grow and interact with biological molecules," says Orme. "While there is a big step between this fundamental research and synthesizing materials that are truly similar to the real thing, we are part of the process to create better materials that affect health."

Pure calcium carbonate in the mineral form called calcite grows only in a symmetrical, six-sided rhombohedral-shape crystal. But that does not explain the intricate shapes found in nature, such as that of seashells. Researchers have known for a long time that organic

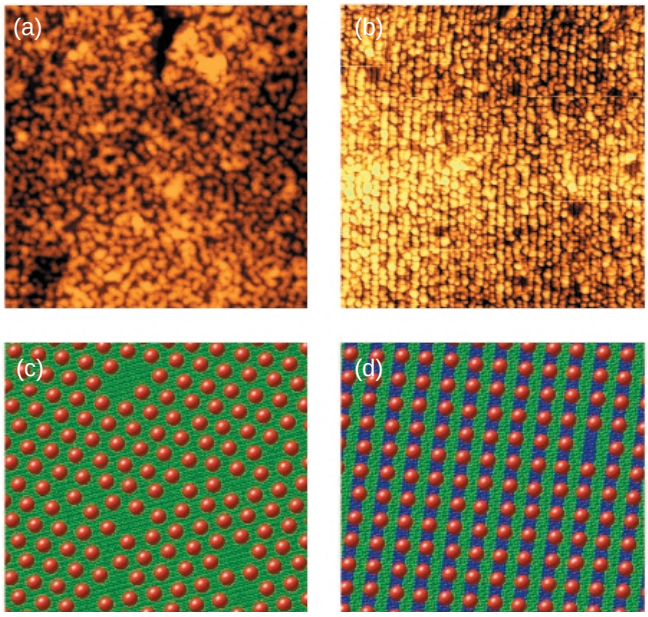


(a) Schematic of dip-pen nanolithography technique. Friction force images of (b) logos, (c) dots drawn on gold, and (d) colloid particles adsorbed preferentially on the dots. Features are composed of 16-mercaptohexadecanoic acid. The lines are 40 to 50 nanometers wide.

molecules can influence the shape of a growing mineral crystal by attaching themselves to it. But it took experiments at Livermore to demonstrate the process in detail, showing how amino acids work at the molecular level to change a growing crystal.

In the experiments, the team added aspartate, one of the more abundant amino acids found in the proteins of shellfish, to calcite crystals growing in solution. Aspartate is typical of many amino acids in that it exhibits handedness, or chirality. As the researchers monitored crystal development, they found that the left-handed and right-handed form of the molecule attached more strongly to opposite atomic steps. The results were crystals that were mirror images of one another. The figure below illustrates how a chiral amino acid influences a growing calcite crystal. By knowing which steps the amino acid interacted with and using the symmetry relations of the crystal and the amino acids, the team was able to predict the binding position of the amino acid to the calcium carbonate step.

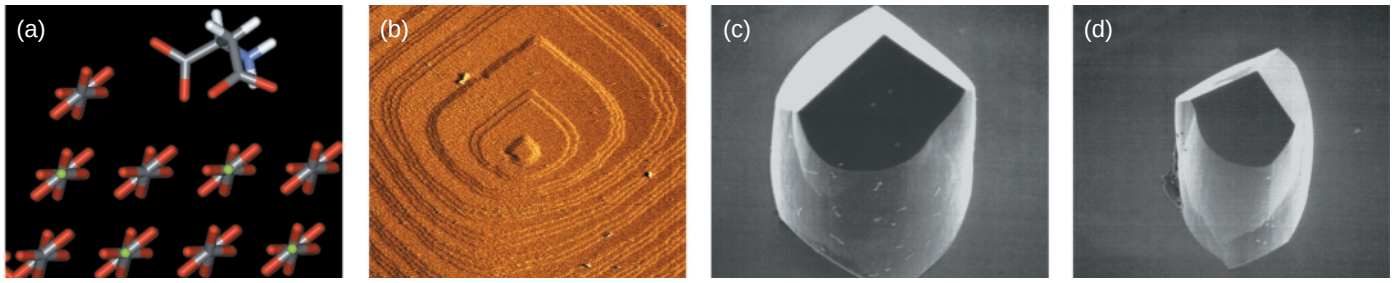
Comparable experiments are just beginning on calcium phosphate, the material used by animals to grow bones. Ultimately, experimental results may be put to myriad uses, from potential laboratory growth of human and animal bones to prevention of scale formation in pipes to the manufacture of toothpaste—any situation in which calcium-based crystals grow naturally or are used.



(a) A homogeneous silica substrate and (b) a nanolaminate of alumina and silica were dipped into a solution of the protein ATCase. Models show that (c) the deposition on the silica surface lacks any linear order, but (d) proteins adsorb to the nanolaminate in ordered rows, indicating the likelihood of growing ordered protien crystals suitable for x-ray diffraction.

Fundamental Science at Work

A nanostructured device is also finding its way into tests for the Yucca Mountain project, the nation's candidate



The interaction of D-aspartic acid (D-Asp) with a calcite mineral surface. (a) Model illustrating the binding of Asp to a calcite step. (b) An atomic force microscope image of calcite steps (0.32 nanometers high) in a solution containing D-Asp. The steps of pure calcite are rhombohedral, but when an Asp-bearing solution is flowed into the fluid cell, the two lower steps interact with Asp and become curved. L-Asp binds more strongly to the left step, and D-Asp binds more strongly to the right step. These differences were used to deduce the binding motif. (c) An electron microscopy image of an approximately 10-micrometer-diameter calcite crystal nucleated on micropatterned, self-assembled monolayers in the presence of D-Asp. The atomic step structure in (b) is reflected in each of the three caps. (d) Crystals nucleated in the presence of L-Asp are mirror images of those nucleated with D-Asp.

for a repository for long-term storage of nuclear wastes. Tests of corrosion-resistant materials are being developed that use patterns formed by "writing" with voltage rather than with chemical inks. A voltage is applied between the AFM tip and a metal or semiconductor substrate to grow oxide patterns under the tip. In the figure below, an oxide greeting is written into a titanium film. The dot on the "i" is made larger and broader by applying a higher voltage. If the nanopatterns blur or dissolve during testing, the change provides a very sensitive indicator that the protective oxide film is changing.

This project is typical of so much fundamental research performed at Livermore. Using funding from the Laboratory Directed Research and Development (LDRD) Program, the oxide templates were originally developed to nucleate calcium phosphate minerals and to control protein deposition onto medical implants. Now, the Yucca Mountain project is putting the template to practical use. Much of the other work at Livermore to grow and image nanostructures also started as basic research, funded either by LDRD or by the Department of Energy's Office of Basic Energy Sciences, before finding a range of applications—including sensors that may someday be a lifesaver.

—Katie Walter

Key Words: atomic force microscope (AFM), biological sensors, biomineralization, carbon nanotubes, chemical sensors, confocal microscope, genomics, nanolaminates, proteomics, surface-enhanced Raman spectroscopy.

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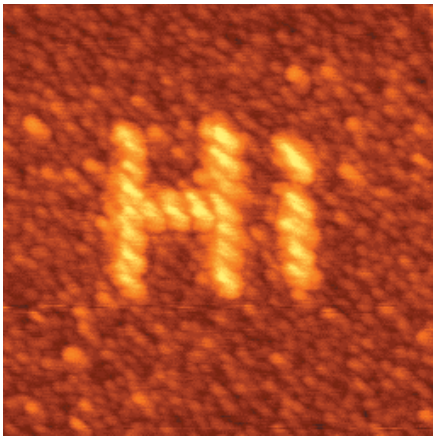

Atomic force microscope image (0.7 micrometers by 0.7 micrometers) of oxide grown on titanium using a voltage applied between the tip of the atomic force microscope and the substrate. (Image made by Livermore summer student researchers R. Sivamani and E. Bochner.)

About the Scientist

CHRISTINE ORME, a physicist in the Materials Science and Technology Division of the Chemistry and Materials Science Directorate, received a B.S. in physics from the University of California at Berkeley. She joined the Laboratory as a postdoctoral fellow after receiving her Ph.D. in physics from the University of Michigan in 1995. Her background is in experimental physics in the area of surface evolution and pattern formation during the growth of thin films. In her thesis work, she combined imaging with kinetic Monte Carlo simulations and continuum modeling to deduce diffusional processes during vapor growth. At Livermore, she uses this background to study crystal growth from solution (rather than from vapor). She is particularly interested in the area of biomineralization where organic molecules substantially change the shape of inorganic crystals; she wants to understand the formation of materials such as shells, bones, and teeth. Recently, she has become interested in the use of electrochemical driving forces to control electrodeposition and corrosive processes, particularly in their application to biomedical implants and corrosion-resistant industrial materials.